Dispatches

Microtubule Motors: LSD2 Trips the Toggle

The Perilipin homologue LSD2 has been identified as a regulator of microtubule motor activity in *Drosophila* embryos. LSD2 is required for the net directional transport of lipid droplets and the new data support a model in which the protein imparts bias onto a molecular toggle that otherwise randomly engages minus and plus end motors in a paired set.

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Microtubule-based motors play a fundamental role in the positioning of molecules and organelles within cells [1]. A number of recent studies indicate that microtubule plus end- and minus end-directed motors — for example, cytoplasmic dynein and kinesin I, respectively — bind their cargoes as an interdependent complex in which the activity of each motor is dependent on the presence of the other [2]. Other proteins in the complex are thought to act as a 'molecular toggle' which ensures that only one motor is active (engaged) at any given time. Net directional transport of cargo toward a particular end of the microtubule is thought to depend on accessory proteins that act on the toggle to favor or sustain activation (engagement) of one motor type over the other [2].

The identification of the first such candidate accessory protein, LSD2, is reported by Welte *et al.* [3] in a recent issue of *Current Biology.* LSD2 is specifically required for the regulated transport of lipid droplets—protein-coated balls of fat essential for efficient energy storage. But LSD2's mode of action appears to be general and its study is likely to provide widespread insights into the mechanisms that regulate motor activity and cargo transport.

LSD2 was identified in a proteomics screen for proteins whose abundance on lipid droplets varies with the transport properties of the droplets. The screen was feasible because lipid droplet transport is highly synchronized in *Drosophila* embryos. A phase of no net transport — equal back and forth motion — is followed in turn by phases of net plus end- and net minus end-directed transport [4] (Figure 1). Moreover, each phase is associated with a distinct embryonic morphology, allowing accurate staging of the organisms prior to droplet purification. The overall protein composition of the lipid droplets does not change during these phases; all the droplets contain the same set of ~400 proteins upon two-dimensional gel analysis [3]. Amongst these proteins, only one — identified by mass spectrometry as LSD2 — exhibited phase-dependent variation in intensity. LSD2 was most abundant on droplets undergoing net plus end-directed transport (phase II droplets). Intermediate amounts of LSD2 protein were found on droplets undergoing net minus end-directed transport (phase III droplets), while only low amounts of protein were found on droplets undergoing no net directional transport (phase I droplets).

Sequence analyses indicated that LSD2 is a member of the PAT (Perilipin, Adipophilin and TIP47) family of proteins, which are conserved in all examined metazoans [5]. Like the other members of this family, LSD2 is abundantly expressed on the surface of lipid droplets [3] and contains the conserved carboxy-terminal PAT domain of unknown function [5]. PAT family members, including LSD2, contain one or more hydrophobic sequence patches, which direct binding to lipid droplets in an as yet undefined way [6]. Perilipin knockout mice have difficulty in forming fat tissue, even when overfed [7,8]. The molecular basis of this defect is not certain, but a number of studies indicate that Perilipin and other PAT family members recruit lipases to lipid droplets to initiate lipid metabolism [7,8]. *Drosophila LSD2* mutants also show defects in lipid droplet metabolism [9], indicating that this is a conserved function of the protein family.

The Welte *et al.* study [3] has revealed a requirement for LSD2

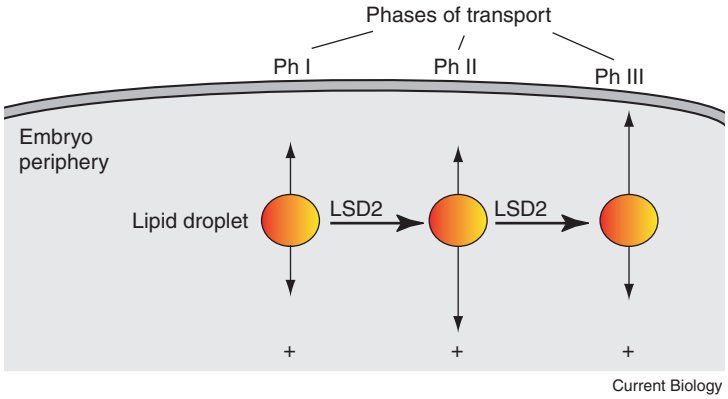

Figure 1. LSD2 is required for the net directional transport of lipid droplets.

The diagram highlights the three phases — Ph I, Ph II and Ph III — of lipid droplet transport in *Drosophila* embryos. The lengths of the arrows correspond to the mean run lengths in the indicated directions, where the plus end of the microtubule is denoted with the +. No bias in mean run lengths in either the minus or plus end direction is seen in the absence of LSD2 (see text).

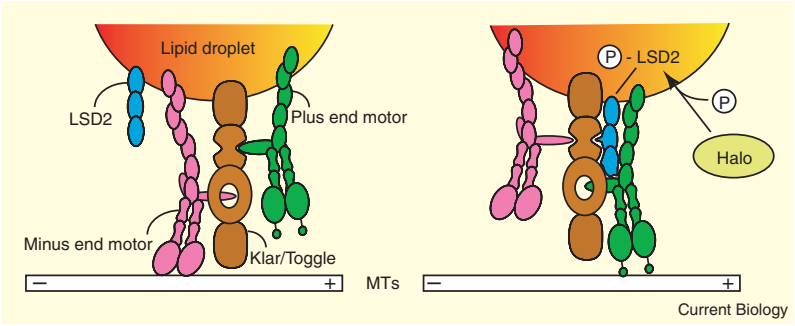

Figure 2. A model to explain how LSD2 might bias motor activity through interactions with the putative molecular toggle, Klar.

(Left panel) In the absence of activated (phosphorylated) LSD2, minus and plus end motors are randomly activated through proposed binding to a single 'activating' site (the donut shaped cavity) on the Klar-containing toggle. Random activation results in short back and forth movements of lipid droplet cargoes, with no net directional transport (see text). The toggle is also proposed to contain a 'quiescent' binding site (the Pacman-shaped cavity) for each motor, preventing promiscuous motor activity that could lead to wasteful tugs-of-war situations. (Right panel) Bias is created within the toggle following phosphorylation of LSD2 by Halo and possibly other kinases (not shown). As indicated in the figure, phosphorylated LSD2 could stabilize or otherwise prolong binding of plus end motors to the activation site of the toggle and/or inhibit binding of such motors to the quiescent site. Other patterns of LSD2 phosphorylation (not shown) could work reciprocally, favoring or sustaining the activation of minus end-directed motors. Thus, the differential biasing of a single toggle device could account for net directional transport toward either the plus or minus end of the microtubule.

in lipid droplet transport. This requirement can be seen at the level of the whole organism by phase contrast microscopy. In wild-type organisms, lipid droplets are transported toward the center of the syncytial embryo just prior to cellularization (during phase II), which results in increased translucence (clearing) of the peripheral cytoplasm [4]. In *LSD2* mutants, the peripheral cytoplasm fails to clear completely and the embryos remain somewhat hazy throughout embryogenesis. The distribution of nuclei, yolk droplets and other cellular structures is normal in *LSD2* mutants, indicating a specific requirement for LSD2 in lipid droplet transport [3].

The clearing of lipid droplets from the peripheral cytoplasm of phase II embryos requires net transport towards the plus ends of microtubules, which are pointed towards the center of the organism (Figure 1). At the level of individual droplets, such transport is seen as a specific increase in the mean run length — uninterrupted travel distance — of plus end-directed transport [10, 11]. Lipid droplets continue to move in both directions during phase II, but the average length of travel in the plus end direction is increased relative to that in the minus end direction (Figure 1A).

This increase is not due to an increase in motor velocity, but rather to an increase in the duration of the run itself. In *LSD2* mutants, mean run lengths are reduced in both directions; however, the reduction is sufficiently greater in the plus end direction to eliminate the normal phase II bias that results in net plus-end transport. As a result, lipid droplets only show back and forth motion in *LSD2* mutant embryos during phase II. Similar back and forth motion is seen during phase III in the *LSD2* mutants, when wild-type droplets exhibit net minus end directed transport (Figure 1). It thus seems that LSD2 is not required for plus end or minus end directed motor activity *per se*, but rather for biasing the activity of one motor type (for example, plus end-directed) over the opposite type, in a stage-specific fashion.

The Welte *et al.* paper [3] provides several tantalizing hints as to how LSD2 might bias one motor activity over another. The first comes from a yeast two-hybrid screen, where LSD2 is shown to interact with Klar, a protein required for the microtubule-directed transport of nuclei, secretory vessels and lipid droplet cargoes [4,12]. *Klar* mutants are not only defective in net directional transport, but also in the normal (non-biased) back and forth movements of cargoes [12]. In *Klar* mutants, the mean distance of back and forth movements is reduced, consistent with the idea that the plus end and minus end motors in a paired set are at war with each other, i.e., simultaneously engaged rather that alternately engaged. This idea is supported by the finding that less force (applied with optical tweezers) is needed to stall cargo transport in *Klar* mutants than in wild-type cells [12]. Together with the finding that Klar is localized to the surface of cargoes [12], these data are consistent with the idea that Klar is itself a component of the toggle that alternately engages plus and minus end motors and that LSD2 biases motor activities through direct interactions with Klar (Figure 2).

The second tantalizing hint as to how LSD2 might control motor activity is provided by the finding that LSD2 is multiply phosphorylated [3]. Different phosphorylated forms of the protein are found in phase I–III embryos suggesting functional relevance with respect to the control of lipid droplet transport. Moreover, in *halo* mutants, where lipid droplets undergo net minus end-directed transport during phase II as well as during phase III [13,14], LSD2 shows a phase III-type phosphorylation pattern throughout embryogenesis. Thus, the ability of LSD2 to bias motor activity (through Klar) would appear to be under the control of Halo, a developmentally regulated kinase (Figure 2). Not surprisingly, kinases have also been implicated in the regulated transport of other cargoes [2], although the mechanistic nature of their influences are not understood.

It will be of interest to determine whether LSD2 binds Klar *in vivo* and to determine whether such binding is influenced, either quantitatively or qualitatively, by the phosphorylation state of

LSD2. It will of course also be of interest to determine how interactions between LSD2 and Klar actually influence Klar in its putative role as a molecular toggle. Does the binding of LSD2 to Klar increase Klar's affinity for one motor type over another (Figure 2)? Or, do LSD2–Klar interactions lead to the recruitment of accessory factors that stabilize or otherwise act on already engaged motors? It will also be of interest to investigate the connection between LSD2-dependent transport and LSD2-dependent metabolism of lipid droplets. One intriguing possibility, suggested by Welte *et al.* [3], is that transport induces structural changes in the droplets that increase the accessibility of their lipid and fat contents to lipases and other metabolic enzymes. Finally, it will be of interest to identify the LSD2-counterparts that control net directional transport of cargoes in other systems.

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Animal Navigation: Northern Exposure

A recent study has found that sparrows moved gradually east above the Arctic Circle completely altered their migration strategy after encountering the massive natural change in declination near the magnetic pole. This should not happen — or should it?

James L. Gould

Juvenile birds regularly migrate thousands of kilometers. Most fly at night, without their parents to guide them. The mystery of how birds manage this apparent impossibility inspires ever-more-heroic attempts to defeat them at this crucial task. In this issue of *Current Biology*, Åkesson and colleagues [1] report a dramatic new way to confound south-bound sparrows: take them along or above the Arctic Circle aboard an icebreaker; fly them to experimental sites by helicopter; and see what directions they choose. Only time will tell whether, once again, the birds have another layer of yet-to-be-deciphered finesse in their navigation repertoire, or if they finally have been driven to the computational wall and are responding in a consistent 'does-not-compute' manner.

Exactly what does a migrating species require? First, the birds need a compass to know which direction they are going, and they come supplied with several: the earth's magnetic field (indicating magnetic north); the celestial pole (indicating true north, about which the stars appear to rotate at night); and the sun's location (as inferred from patterns of sun-centered polarization which, with a suitable time sense, also specifies true north). Second, they need to know at least roughly — and in some instances quite precisely — where they are relative to their goal. In the case of homing pigeons, this ability is known as a map sense, and has a resolution of a very few kilometers [2].

The map sounds quite mysterious compared to the compass, but they are both daunting challenges. Consider the problem from the bird's point of view. First of all, it is cloudy a lot, so much of the time you can forget about using celestial cues. But then, why not just use magnetic north? If you are born at a high latitude — where large numbers of species breed — there is often a large discrepancy between magnetic and true north — the declination error, which arises in part from the 1400 kilometer separation of this point from the geographic pole. Worse, declination generally changes as you fly south. And even if the evening is clear, the stars and patterns of polarized light change with both latitude and date.

Birds dispose of these problems by periodically calibrating one compass against the other [3,4]. Recent evidence has shown that when the sky is